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16. ABSTRACT

This report summarizes and evaluates information relevant to a preliminary interim assessment of adverse health effects associated with specific chemicals or compounds. The Office of Emergency and Remedial Response (Superfund) uses these documents in preparing cost-benefit analyses under Executive Order 12991 for decision-making under CERCLA. All estimates of acceptable intakes and carcinogenic potency presented in this document should be considered as preliminary and reflect limited resources allocated to this project. The intent in these assessments is to suggest acceptable exposure levels whenever sufficient data are available. The interim values presented reflect the relative degree of hazard associated with exposure or risk to the chemical(s) addressed. Whenever possible, two categories of values have been estimated for systemic toxicants (toxicants for which cancer is not the endpoint of concern). The first, RfD_S or subchronic reference dose, is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs during a limited time interval. The RfD is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs for a significant portion of the lifespan. For compounds for which there is sufficient evidence of carcinogenicity, q_1^*s have been computed, if appropriate, based on oral and inhalation data if available.

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PREFACE

This report summarizes and evaluates information relevant to a preliminary interim assessment of adverse health effects associated with benzidine. All estimates of acceptable intakes and carcinogenic potency presented in this document should be considered as preliminary and reflect limited resources allocated to this project. Pertinent toxicologic and environmental data were located through on-line literature searches of the TOXLINE, CANCERLINE and the CHEMFATE/DATALOG data bases. The basic literature searched supporting this document is current up to May, 1986. Secondary sources of information have also been relied upon in the preparation of this report and represent large-scale health assessment efforts that entail extensive peer and Agency review. The following Office of Health and Environmental Assessment (OHEA) sources have been extensively utilized:

U.S. EPA. 1978. Review of the Environmental Effects of Pollutants. II. Benzidine. Health Effects Research Laboratory, U.S. EPA, Cincinnati, OH. EPA 600/1-78-024. NTIS PB281076.

U.S. EPA. 1980a. Ambient Water Quality Criteria for Benzidine. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulations and Standards, Washington, DC. EPA 440/5-80-023. NTIS PB81-117342.

U.S. EPA. 1983a. Reportable Quantity Document for Benzidine. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC.

U.S. EPA. 1986. Health and Environmental Effects Profile for Benzidine. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

The intent in these assessments is to suggest acceptable exposure levels whenever sufficient data were available. Values were not derived or larger uncertainty factors were employed when the variable data were limited in scope tending to generate conservative (i.e., protective) estimates. Nevertheless, the interim values presented reflect the relative degree of hazard associated with exposure or risk to the chemical(s) addressed.

Whenever possible, two categories of values have been estimated for systemic toxicants (toxicants for which cancer is not the endpoint of concern). The first, RfD_S (formerly AIS) or subchronic reference dose, is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs during a limited time interval (i.e., for an interval that does not constitute a significant portion of the lifespan).

This type of exposure estimate has not been extensively used, or rigorously defined, as previous risk assessment efforts have been primarily directed towards exposures from toxicants in ambient air or water where lifetime exposure is assumed. Animal data used for RfD_S estimates generally include exposures with durations of 30-90 days. Subchronic human data are rarely available. Reported exposures are usually from chronic occupational exposure situations or from reports of acute accidental exposure. These values are developed for both inhalation (RfD_{SI}) and oral (RfD_{SO}) exposures.

The RfD (formerly AIC) is similar in concept and addresses chronic exposure. It is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs for a significant portion of the lifespan [see U.S. EPA (1980a,b) for a discussion of this concept]. The RfD is route-specific and estimates acceptable exposure for either oral (RfD_O) or inhalation (RfD_I) with the implicit assumption that exposure by other routes is insignificant.

Composite scores (CSs) for noncarcinogens have also been calculated where data permitted. These values are used for identifying reportable quantities and the methodology for their development is explained in U.S. EPA (1983).

For compounds for which there is sufficient evidence of carcinogenicity RfD_S and RfD values are not derived. For a discussion of risk assessment methodology for carcinogens refer to U.S. EPA (1980b). Since cancer is a process that is not characterized by a threshold, any exposure contributes an increment of risk. For carcinogens, q_1^*s have been computed, if appropriate, based on oral and inhalation data if available.

ABSTRACT

In order to place the risk assessment evaluation in proper context, refer to the preface of this document. The preface outlines limitations applicable to all documents of this series as well as the appropriate interpretation and use of the quantitative estimates presented.

Benzidine has been implicated as a human carcinogen U.S. EPA Group A, in workers exposed in the manufacturing process (Zavon et al., 1973). In this study, 13/25 workers exposed for an average of 11.46 years developed cancer of the urinary bladder. The U.S. EPA (1980a) derived an estimate of oral carcinogenic potency from this study of 234.13 $(mg/kg/day)^{-1}$. An estimate of carcinogenic potency of 111 $(mg/kg/day)^{-1}$ was derived from the same study for inhalation exposure.

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LIST OF ABBREVIATIONS

B	Potency factor
BCF	Bioconcentration factor
$-Bd^t$	Increased cancer incidence with age
BUN	Blood urea nitrogen
CAS	Chemical Abstract Service
CS	Composite score
DMBA	Dimethyl benzanthracene
K_{oc}	Soil sorption coefficient standardized with respect to organic matter
MED	Minimum effective dose
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
ppm	Parts per million
RfD	Reference dose
RfD_I	Inhalation reference dose
RfD_O	Oral reference dose
RfD_{SI}	Subchronic inhalation reference dose
RfD_{SO}	Subchronic oral reference dose
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
SGOT	Serum glutamic oxaloactic transaminase
SGPT	Serum glutamic pyruvic transaminase
TLV	Threshold limit value
TWA	Time-weighted average

1. ENVIRONMENTAL CHEMISTRY AND FATE

Selected chemical and physical properties and environmental fate of benzidine are listed in Table 1-1.

In the atmosphere, benzidine is probably removed rapidly by reaction with OH radical. Based on reaction kinetics (Atkinson, 1985) of structurally similar compounds (aniline and biphenyl), the half-life for the benzidine reaction with OH radical is expected to be ~1 day. Photolysis and direct oxidation with oxygen atoms may also contribute to the removal of benzidine from the atmosphere.

A persistence study conducted with a single lake water found that benzidine had a half-life of 4 hours (Howard and Saxena, 1976). In aquatic systems, oxidation reactions with metal cations, peroxy radicals and oxygen are probably the most important fate processes for benzidine. The estimated peroxy radical reaction half-life is 6.3 hours (Mabey et al., 1981). Photosensitized photolysis of benzidine or photolysis of benzidine-oxidation complexes in water may also have some importance (U.S. EPA, 1981; Callahan et al., 1979). Benzidine is rapidly and strongly adsorbed to clay particles (Callahan et al., 1979; Furukawa and Bindley, 1973; Lahav and Raziel, 1971; U.S. EPA, 1981) and by sediment (Zierath et al., 1980) indicating adsorption in aquatic systems may be significant.

In clay silty loam, ~70% disappearance of benzidine was observed after 1 week (Lu et al., 1975). Benzidine will adsorb to soil to form complexes with clay particles and oxidize by reaction with metal cations (NLM, 1986).

TABLE 1-1

Selected Chemical and Physical Properties and Half-Lives for Benzidine

Property	Value	Reference
CAS number:	92-87-5	
Chemical class:	aromatic diamine	
Molecular weight:	184.24	
Vapor pressure:	5×10^{-4} (estimated at unspecified temperature)	Mabey et al., 1981
Water solubility:	276 mg/ℓ at 20°C	Schmit-Bleek et al., 1982
	520 mg/ℓ at 25°C	Shriner et al., 1978
Log octanol/water partition coefficient:	1.34	Hansch and Leo, 1985
Ionization constant:	$pK_1 = 4.66$ at 30°C	Weast, 1985
	$pK_2 = 3.57$ at 30°C	Weast, 1985
Bioconcentration factor:	110 fish (*Gambusia affinis*)	Lu et al., 1975
	83 Golden orfe (*Leuciscus idus*)	Shriner et al., 1978
	44 bluegill sunfish (*Lepomis macrochirus*)	
Soil adsorption coefficient (K_{oc}):	264-82,110 (depending upon pH and soil surface area)	Zierathe et al., 1980
Half-lives:		
Air	~1 day	Atkinson, 1985
Water	4 hours	Howard and Saxena, 1976
Soil	~ days	Lu et al., 1975

The ionic sorption and subsequent oxidation will increase with the lowering of pH and increase of clay containing metal cations capable of catalyzing the oxidation reaction (Callahan et al., 1979; Zierath et al., 1980). Photolysis on sunlit soil surfaces may also occur (Lahav and Raziel, 1971; Kotzias et al., 1981). Based on sorption coefficients in 14 different soils and sediments (Zierath et al., 1980), benzidine is not expected to leach significantly in most types of soil.

2. ABSORPTION IN HUMANS AND EXPERIMENTAL ANIMALS

2.1. ORAL

Lynn et al. (1984) administered ^{14}C-benzidine orally or intravenously to male F344 rats and measured the radioactivity excreted in urine and feces. At doses of 0.5-5.0 mg/kg, fecal excretion at 24 hours was nearly identical at 50.4-54.5% following either route of administration. These data suggest that gastrointestinal absorption of benzidine is very rapid and virtually complete, at least in rats. The recovery of ~50% of the administered radioactivity from the feces was attributed to biliary excretion rather than to lack of absorption. This was confirmed by the recovery from the bile of bile duct cannulated rats of 71.2% of the radioactivity associated with an intravenous dose of ^{14}C-benzidine within 3 days of treatment.

2.2. INHALATION

Little information concerning the inhalation absorption of benzidine could be located in the available literature. Absorption by inhalation can be inferred from systemic toxicity and carcinogenicity observed in humans and animals following inhalation exposure (see Chapters 3 and 4). Ghetti (1960) observed unspecified amounts of benzidine or its metabolites in the urine of dogs following inhalation exposure. As reported in an abstract of a Polish study by Krajewska et al. (1980), the mean annual dose of benzidine absorbed by workers was 0.065 mg. Only 10% of the dose was attributable to inhalation exposure.

3. TOXICITY IN HUMANS AND EXPERIMENTAL ANIMALS

3.1. SUBCHRONIC

3.1.1. Oral. Frith and Dooley (1976) gave groups of 24 male and 24 female 3-week-old Balb/c mice benzidine dihydrochloride in their drinking water at levels of 0, 50, 150, 300 and 500 ppm for 8, 19 and 39 weeks. A dose-related increase in the incidence and severity of mild periportal fibrosis, infiltration of lymphocytes and deposits of brown pigment (suggestive of ceroid in the liver) was observed presumably at $\geq$50 ppm after 8, 19 or 39 weeks.

Boyland et al. (1954) fed groups of 10 female Wistar rats diets containing 0 or 0.017% benzidine by weight (170 ppm) until a tumor appeared or death occurred. All treated rats died within 93-224 days. Most of the treated rats developed fatty degeneration and regeneration of the bile ducts and fatty degeneration of the liver.

In a study to determine the MTD for long-term cancer studies, a 6-week dose tolerance test was conducted in which unspecified numbers of male and female B6C3F1 mice were fed diets containing benzidine dihydrochloride in concentrations of 100, 200, 400, 600 and 800 ppm (Vesselinovitch et al., 1975; Rao et al., 1971). A significant dose-related weight loss of 5, 10 and 18% of initial body weight was observed in males fed 400, 600 and 800 ppm benzidine dihydrochloride, respectively, by the end of the experiment. A less pronounced weight loss of 7.5% was observed in females at the highest dose. A dose-related increased severity of histopathological lesions which included cloudy swelling of the liver, vacuolar degeneration of the renal tubules and hyperplasia of myeloid elements in the bone marrow and lymphoid cells in the spleen and thymic cortex were observed but it was not clear if these lesions occurred at all doses.

3.1.2. Inhalation. Subchronic inhalation toxicity data could not be located in the available literature.

3.2. CHRONIC

3.2.1. Oral. Littlefield et al. (1983, 1984) gave groups of 72-120 male mice of two strains and 72-120 female mice of two strains benzidine dihydrochloride in drinking water for ~33 months (see Table 4-1 for detailed strain and dose data). Mice of both strains and sexes exhibited a significantly increased incidence of hemosiderosis of the spleen at 120 ppm as compared with controls. Females of both strains exhibited liver cytological alterations (acidophilic, basophilic, vacuolated and mixed cell types) at concentrations ≥20 ppm. Hyperplasia of the bile duct was observed in females of both strains at concentrations ≥40 ppm and in both strains of males at 160 ppm. Megakaryocytosis of the bone marrow was observed at concentrations ≥60 ppm in female mice and ≥120 ppm in male mice. Brain vacuolization was observed in all treatment groups. Atrophy of the ovary was observed in F_1 mice at 30 ppm and in MC mice at ≥40 ppm. The carcinogenic effects observed in this study are discussed in Section 4.2.1.

3.2.2. Inhalation. Zabezhinskii (1970) exposed a group of 48 noninbred albino rats of both sexes, weighing 100-120 g, to benzidine by inhalation at a concentration of 10-20 mg/m³ (0.755-1.5 mg/kg/day), 4 hours/day, 5 times/week for up to 28 months. Controls were exposed to "pure air." After 11 months of exposure, liver cirrhosis was observed.

3.3. TERATOGENIC AND OTHER REPRODUCTIVE EFFECTS

Very little information concerning the teratogenicity of benzidine was located. Vesselinovitch et al. (1979) observed an increased incidence of hepatocellular carcinomas in the male offspring of female mice maintained on a diet containing 150 ppm benzidine dihydrochloride during gestation. Female offspring were not affected.

3.4. TOXICANT INTERACTIONS

Data concerning toxicant interactions could not be located in the available literature.

4. CARCINOGENICITY

4.1. HUMAN DATA

4.1.1. Oral. Human oral carcinogenicity data could not be located in the available literature.

4.1.2. Inhalation. Zavon et al. (1973) observed 13/25 workers (average age = 56.5 years) exposed to benzidine primarily by inhalation at levels of <0.005-17.6 mg/m³ for ~11.46 years had bladder cancer. Metastases to the neck and abdomen were noted in two workers; three workers also had four renal tumors. Eleven of the thirteen cases were attributed to benzidine exposure alone. From a mean concentration of ~0.035 mg/L benzidine in the urine of the exposed workers, U.S. EPA (1980a) estimated a mean total accumulated dose of 130 mg/kg. Several limitations of the study were noted, including the absence of controls. The number of tumors expected was not reported, preventing relative risk from being calculated. (Using "Third National Cancer Survey: Incidence Data," however, a relative risk of ~100 can be estimated.) Smoking and exposure to other chemicals was not controlled, but because of the unusually high apparent oncogenic potency of benzidine these may not be major contributing factors (NCI, 1975).

Tsuchiya et al. (1975) observed 72 bladder cancer cases (21 were fatal) in 1015 benzidine production and use workers. The incidence of bladder cancer in production workers was 61/346 (17.6%) and in use workers was 11/669 (1.6%). No control population was included in the study design and relative risk could not be calculated because the number of tumors expected was not reported.

Meigs et al. (1986) conducted a 30-year follow-up study on the incidence of bladder tumors among workers in Connecticut exposed to benzidine. There was a statistically significant excess of bladder tumors among male cohort

members which was confined to those males with the highest estimated level of benzidine exposure. Exposure was estimated as high, medium or low according to the length of individual exposure to benzidine (≥2 years = high; 6 months to 2 years = medium; ≤6 months = low). Among the 830 males, a total of 48 first primary cancers were reported; of these 48, eight cases of bladder cancer were found. Benzidine exposure was present in 7 (6 high; 1 medium) of the 8 cases of bladder cancer. No significantly elevated risks were found for cancers at other anatomic sites in men or at any anatomic site for women; nor was there any pattern of increasing risk with increasing benzidine exposure for site other than bladder. In addition, the elevated bladder cancer was greater for men first employed during the earliest years of the plant, namely, 1945-1949. Smoking histories were available for 21 of the 48 primary cancers; of the 8 cases of bladder cancer, 3 were long-term cigarette smokers.

Numerous case reports were cited in the available literature concerning the occurrence of bladder cancer in dyestuff industry workers. These reports provide qualitative evidence of the carcinogenic effect of benzidine. Several sources that contain reviews of these studies are IARC (1972, 1982), U.S. EPA (1980a,b), ACGIH (1980) and NIOSH (1978). Haley (1975), Hamilton and Hardy (1974) and Parkes (1976) reviewed the history of benzidine-induced cancer in humans. Haley (1975) also discussed the international dimensions of benzidine-induced cancer. Several early reports came from industrial settings in which workers were exposed to benzidine alone. Aboulker and Smagghe (1953) reported 21 cases of bladder tumors in workers at a dyestuff plant. In two of these cases, the workers were exposed to benzidine alone. Uebelin and Pletscher (1954) observed 100 cases of urinary tract tumors in Swiss dyestuff workers. Twenty of these workers were

exposed to benzidine alone. Duration of exposure was ~11.6 years (range, 1-29 years). The mean induction period was 14.8 years (range 5-29 years). Billiard-Duschesne (1960) described 12 bladder cancer cases using workers at a plant where the only aromatic amine manufactured was benzidine. Goldwater et al. (1965) reported 17 cases of bladder cancer in a population of 76 men occupationally exposed to benzidine only for up to 33 years, and commented that this incidence may be deceptively low since many workers quit their jobs after the first examination. Ferber et al. (1976) reported that 36 cases of bladder tumors occurred in workers exposed to benzidine alone during the period of 1930-1975.

4.2. BIOASSAYS

4.2.1. Oral. Littlefield et al. (1983, 1984) studied the carcinogenicity of benzidine in two strains of male and female mice (Table 4-1). Groups of 72-120 male mice of each strain and groups of 72-120 female mice of each strain were exposed to benzidine dihydrochloride in drinking water for ~33 months. Strain and dosage data and results are presented in Table 4-1. The incidence of hepatocellular carcinomas increased with increased dose, with the exception of MC males whose hepatocellular carcinoma incidence reached a maximum of 37/71 at 120 ppm and diminished to 32/71 at 160 ppm. Time to tumor was decreased as demonstrated by F_1 females (representative of trends for both strains and sexes) in which only a mean of 464 days was required for tumor formation as compared with 1023 days for controls. Littlefield et al. (1983) also reported treatment-related increased incidences of adenoma of the Harderian gland and uterine angioma. Since these incidences were much lower than those reported for hepatocellular carcinomas, they will not be considered here.

TABLE 4-1

Incidence of Hepatocellular Carcinoma in Two Strains of Mice Given 100% Benzidine Dihydrochloride in Drinking Water for ~33 Months[a]

Strain[b]	Sex	Concentration (ppm)	Corresponding Dose (mg/kg/day)[c]	Tumor Incidence (%)
F_1	M	0	0	14/125 (11)
F_1	M	30	3.7	24/119 (20)
F_1	M	40	4.7	30/96 (31)
F_1	M	60	7.5	23/71 (32)
F_1	M	80	9.1	35/71 (49)
F_1	M	120	13.7	51/71 (72)
F_1	M	160	16.6	49/71 (69)
F_1	F	0	0	3/124 (2)
F_1	F	20	2.6	51/120 (43)
F_1	F	30	4.0	52/95 (55)
F_1	F	40	5.2	45/72 (63)
F_1	F	60	8.1	55/71 (77)
F_1	F	80	11.2	60/69 (87)
F_1	F	120	22.3	64/72 (89)
MC	M	0	0	17/123 (14)
MC	M	30	3.5	20/118 (17)
MC	M	40	4.7	20/95 (21)
MC	M	60	7.0	23/72 (32)
MC	M	80	9.4	24/71 (34)
MC	M	120	13.2	37/71 (52)
MC	M	160	16.4	32/71 (45)
MC	F	0	0	10/125 (8)
MC	F	20	2.5	54/119 (45)
MC	F	30	3.8	43/95 (45)
MC	F	40	5.1	31/71 (44)
MC	F	60	7.8	37/72 (51)
MC	F	80	10.0	51/69 (74)
MC	F	120	14.8	56/72 (78)

[a]Source: Littlefield et al., 1984

[b]F_1 = hybrids from crossing BALB/c males to C57BL females; MC = monohybrid cross offspring of mating F_1 females with F_1 males

[c]Dose in mg/kg/day calculated from data provided by investigators

Frith et al. (1979, 1980) exposed groups of male and female weanling mice (Table 4-2) to drinking water containing 0, 30, 50, 120, 200 or 400 ppm benzidine dihydrochloride for 40, 60 or 80 weeks. Mice that died before 30 weeks (12 females and 22 males) were not included in the data analysis; these mice had no liver lesions. The ratio of hepatocellular carcinomas to adenomas increased with increased dose and longer length of administration in female mice. The ratio of hepatocellular carcinomas increased with increased length of administration, but not with dose in male mice. Frith et al. (1980) suggested a sequential morphogenesis from cellular alteration to adenoma to carcinoma and reported that the incidence of pulmonary metastases increased with increased length of administration.

Frith and Dooley (1976) gave groups of 24 male and 24 female Balb/c, 3-week-old mice benzidine dihydrochloride in drinking water at levels of 0, 50, 150, 300 and 500 ppm. After 8, 19 and 39 weeks of exposure, groups of 4, 4 and 16 animals of each sex, respectively, were killed for comprehensive gross and microscopic examination of a wide variety of organs and tissues. The incidence of tumors in the 300 and 500 ppm groups at 8 or 19 weeks or in the control, 50 or 100 ppm groups was not reported. Presumably, no tumors were found in these groups at these times. Liver tumors were observed in 300 and 500 ppm mice at 39 weeks; the incidence of tumors was 1/12 and 3/7 in males and 1/9 and 3/12 in females in the 300 and 500 ppm dose groups, respectively.

In a series of experiments, Vesselinovitch et al. (1975) administered 50 or 100 ppm benzidine dihydrochloride in the diet to groups of 50 male and 50 female B6C3F1 mice for 90 weeks. Presumably, a control group was maintained on a diet without benzidine dihydrochloride, but this was not explicitly reported. The authors reported that complete autopsies were performed but

TABLE 4-2

Incidence of Liver Tumors in a Drinking Water Study of Pure Benzidine Dihydrochloride in Mice[a,b]

Sex	Concentration (ppm)	Duration of Treatment and Study (weeks)	Tumor Type	Tumor Incidence (%)
F	0	40	adenoma	0/96 (0)
F	30	40	adenoma	2/195 (1)
F	60	40	adenoma	1/144 (1)
F	120	40	adenoma	2/100 (2)
F	200	40	adenoma	7/100 (7)
F	400	40	adenoma	13/55 (24)
F	0	60	adenoma	1/96 (1)
F	30	60	adenoma	7/146 (5)
F	60	60	adenoma	9/106 (8)
F	120	60	adenoma	17/114 (15)
F	200	60	adenoma	15/121 (12)
F	400	60	adenoma	2/79 (3)
F	0	80	adenoma	0/95 (0)
F	30	80	adenoma	9/86 (10)
F	60	80	adenoma	11/85 (13)
F	120	80	adenoma	5/69 (7)
F	200	80	adenoma	1/17 (6)
F	400	80	adenoma	0/7 (0)
M	0	40	adenoma	0/99 (0)
M	30	40	adenoma	0/199 (0)
M	60	40	adenoma	0/143 (0)
M	120	40	adenoma	1/100 (1)
M	200	40	adenoma	2/102 (2)
M	400	40	adenoma	1/55 (2)
M	0	60	adenoma	0/96 (0)
M	30	60	adenoma	2/142 (1)
M	60	60	adenoma	3/95 (3)
M	120	60	adenoma	9/98 (9)
M	200	60	adenoma	6/90 (7)
M	400	60	adenoma	7/49 (14)

TABLE 4-2 (cont.)

Sex	Concentration (ppm)	Duration of Treatment and Study (weeks)	Tumor Type	Tumor Incidence (%)
M	0	80	adenoma	2/91 (2)
M	30	80	adenoma	2/85 (2)
M	60	80	adenoma	6/90 (7)
M	120	80	adenoma	11/89 (12)
M	200	80	adenoma	5/40 (13)
M	400	80	adenoma	4/37 (11)
F	0	40	carcinoma	0/96 (0)
F	30	40	carcinoma	0/195 (0)
F	60	40	carcinoma	0/144 (0)
F	120	40	carcinoma	1/100 (1)
F	200	40	carcinoma	4/100 (4)
F	400	40	carcinoma	10/55 (19)
F	0	60	carcinoma	1/96 (1)
F	30	60	carcinoma	3/146 (2)
F	60	60	carcinoma	7/106 (7)
F	120	60	carcinoma	33/114 (29)
F	200	60	carcinoma	86/121 (71)
F	400	60	carcinoma	72/79 (91)
F	0	80	carcinoma	0/95 (0)
F	30	80	carcinoma	12/86 (14)
F	60	80	carcinoma	32/85 (38)
F	120	80	carcinoma	60/69 (87)
F	200	80	carcinoma	15/17 (88)
F	400	80	carcinoma	6/7 (86)
M	0	40	carcinoma	0/99 (0)
M	30	40	carcinoma	1/199 (1)
M	60	40	carcinoma	0/143 (0)
M	120	40	carcinoma	0/100 (0)
M	200	40	carcinoma	1/102 (1)
M	400	40	carcinoma	1/55 (2)
M	0	60	carcinoma	1/95 (1)
M	30	60	carcinoma	1/142 (1)
M	60	60	carcinoma	4/95 (4)
M	120	60	carcinoma	8/98 (9)
M	200	60	carcinoma	11/90 (12)
M	400	60	carcinoma	12/49 (24)

TABLE 4-2 (cont.)

Sex	Concentration (ppm)	Duration of Treatment and Study (weeks)	Tumor Type	Tumor Incidence (%)
M	0	80	carcinoma	0/91 (0)
M	30	80	carcinoma	5/85 (6)
M	60	80	carcinoma	7/90 (8)
M	120	80	carcinoma	16/89 (18)
M	200	80	carcinoma	10/40 (25)
M	400	80	carcinoma	23/37 (62)

[a]Source: Frith et al., 1979, 1980

[b]F_1 (C57B1 females x Balb/c males) and MC (F_1 females x F_1 males) mice combined. Incidence data for the two strains were combined because there were no statistical differences between the two strains.

did not provide sufficient detail for a determination of the adequacy of the gross and microscopic examination. The MTD was estimated by pretesting the mice. Vesselinovitch et al. (1975) also conducted gavage studies in mice. Groups of 75 male and 75 female B6C3F1 mice were gavaged twice weekly with doses of 0.5 or 1.0 mg benzidine/mouse at each intubation for 90 weeks. These intermittent doses were considered to be equivalent to the weekly intake of benzidine dihydrochloride by the mice exposed continuously to 50 and 100 ppm in the diet (Table 4-3). Higher incidences of liver tumors and Harderian gland tumors were observed in the mice treated continuously in the diet compared with the mice receiving intermittent equivalent gavage doses. The incidence of lung adenomas, however, was higher in the mice treated by gavage than in the mice treated by dietary administration.

In addition to the tumors presented in Table 4-3, a marginally increased incidence of lymphoreticular tumors were observed in the treated mice compared with controls. (This was the only mention of controls in the entire report.) The incidences in individual groups were low; therefore, Vesselinovitch et al. (1975) reported the combined incidences regardless of dose or sex to be 19/200 (9.5%) for the continuously (diet) exposed mice; 17/300 (5.7%) for the intermittently (gavage) exposed mice and 4/194 (2.0%) for the controls.

Griswold et al. (1968) exposed groups of twenty 40-day-old female Sprague-Dawley rats to a total of 12, 25, 35 and 50 mg benzidine/rat by gavage in sesame oil, given in 10 doses at 3-day intervals over a 30-day treatment period. Controls were dosed with sesame oil alone. The rats were observed for 9 months before sacrifice. Gross lesions were recorded, and the pituitary, adrenals, kidneys, spleen and liver were weighed. In addition to diseased tissues, the mammary, intestinal tract, pituitary, liver,

TABLE 4-3

Incidence of Tumors in B6C3F1 Mice Treated Orally with Benzidine Dihydrochloride (Certified Grade)*

Sex	Concentration or Dose	Duration of Treatment (weeks)	Duration of Study (weeks)	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence
M	50 ppm	90	90	dietary	liver harderian gland lung	carcinoma NR adenomas	3/50 9/50 2/50
M	100 ppm	90	90	dietary	liver harderian gland lung	carcinoma NR adenomas	11/50 18/50 3/50
F	50 ppm	90	90	dietary	liver harderian gland lung	carcinoma NR adenomas	13/50 3/50 2/50
F	100 ppm	90	90	dietary	liver harderian gland lung	carcinoma NR adenomas	32/50 3/50 2/50
M	0.5 mg twice weekly	90	90	NR/gavage	liver harderian gland lung	carcinoma NR adenomas	3/75 2/75 12/75
M	1.0 mg twice weekly	90	90	NR/gavage	liver harderian gland lung	carcinoma NR adenomas	12/75 11/75 19/75
F	0.5 mg twice weekly	90	90	NR/gavage	liver harderian gland lung	carcinoma NR adenomas	4/75 2/75 5/75

TABLE 4-3 (cont.)

Sex	Concentration or Dose	Duration of Treatment (weeks)	Duration of Study (weeks)	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence
F	1.0 mg twice weekly	90	90	NR/gavage	liver harderian gland lung	carcinoma NR adenomas	17/75 5/75 4/75
M	150 ppm	39 54 84	84	dietary	liver liver liver	carcinoma carcinoma carcinoma	35/50 25/50 22/50

QUALITY OF EVIDENCE

Strengths of Study: Both sexes of one species (mice) were dosed at several levels by a relevant route of administration over their lifespan. The MTD was determined by pretesting.

Weakness of Study: Tumor incidence was not always expressed as number of tumors/number of animals treated. Insufficient information was given to assess the adequacy of the histopathologic examination. Only x^2 test was used to determine statistical significance between various experimental groups. With the exception of one table, no data for controls were reported.

Overall Adequacy: Limited

*Source: Vesselinovitch et al., 1975

NR = Not reported

0091h

12/29/86

ovaries and adrenals were prepared for histological examination. The 12, 25 and 50 mg dosage group had carcinomas and adenomas of the mammary glands (Table 4-4). There were no survivors in the 35 mg group at 9 months. The authors noted spontaneous regression of some of the palpable masses; therefore, some tumors were not present at autopsy.

4.2.2. Inhalation. Zabezhinskii (1970) exposed a group of 48 noninbred albino rats of both sexes, weighing 100-120 g, to benzidine by inhalation at a concentration of 10-20 mg/m³, 4 hours/day, 5 times/week for 28 months. Control rats were exposed to "pure air." Myeloid leukemia developed in five rats after 13, 22, 24, 25 and 28 months of the experiment. Mammary gland fibroadenomas occurred in 2/28 rats, and mammary gland carcinomas occurred in 2/28 rats, one of which was found in a male rat. A hepatoma was observed in one rat. These tumor types appeared in 8/28 rats; presumably, more than one tumor type was found in some of the rats. The incidence of mammary adenomas in control rats was 2/21.

4.3. OTHER RELEVANT INFORMATION

Several other oral exposure studies were conducted with benzidine dihydrochloride in mice by Vesselinovitch et al. (1975, 1979). Although insufficiently reported for consideration in the quantitative estimation of carcinogenic potential, these reports qualitatively indicate the carcinogenicity of benzidine. In 84-90 week studies in B6C3F1 mice, dietary levels of 100-150 ppm and gavage doses of 1.0 mg twice weekly resulted in increased incidences of liver carcinoma, Harderian gland tumors and lung adenomas. In mice fed a diet containing 150 ppm benzidine hydrochloride for 39, 54 or 84 weeks and killed at 90 weeks of age. The incidence of liver carcinomas at termination was inversely related to length of exposure. When mice were fed

TABLE 4-4

Incidence of Mammary Gland Tumors in Female Sprague-Dawley Rats Treated by Gavage With Benzidine (Pure) in Sesame Oil for 30 Days and Observed for 9 Months[a]

Dose[b] (mg/rat)	Tumor Type	Tumor Incidence (%)
50	carcinoma	4/5 (80)
35	no survivors at 9 months	NA
25	carcinoma	7/9 (78)
12	carcinoma, adenoma	5/10 (50)
0	carcinoma, adenoma, hyperplasia	5/132 (4)

QUALITY OF EVIDENCE

Strengths of Study: Several doses were administered by a relevant route of administration (oral); the MTD was estimated through pretests.

Weakness of Study: Only female rats of one species were used; dosing occurred at 3-day intervals over a 30-day period, which is a period substantially less than the lifespan of a rat; histopathology was not comprehensive and the data were not subjected to statistical analysis.

Overall Adequacy: Limited

[a]Source: Griswold et al., 1968

[b]10 doses at 3-day intervals; dose reported = total dose

NA = Not applicable

the test diet from 6-45 weeks of age followed by control diet until terminations at 45, 60, 75 or 90 weeks of age, the incidence of total liver tumors increased with increasing duration of lifespan. The author concluded that "once carcinogenesis has been initiated, the neoplastically-committed cells express their malignant character once a sufficient observational period has been allowed." In experiments with dietary benzidine dihydrochloride in the prenatal and adult periods, it appeared that younger animals were more susceptible to a carcinogenic response.

Increased incidence of hepatomas were observed in ICR/SCL mice fed dietary benzidine at 500-100 ppm (Osanai, 1976; Ikuyama et al., 1978). By varying the amount of forced exercise to which the mice were subjected, Ikuyama et al. (1978) concluded that exercise decreased the carcinogenic response.

Other species of animals appear to form other types of tumors following oral exposure to benzidine. Mammary tumors including carcinomas developed in male and female rats treated with benzidine by gavage (Griswold et al., 1968; Zabezhinskii, 1967). Cholangiomas as well as hepatomas were increased in Syrian golden hamsters exposed to benzidine or benzidine dihydrochloride for life (Saffiotti et al., 1967; Sellakumar et al., 1969).

Numerous intraperitoneal, intramuscular and subcutaneous studies yielding positive results were summarized in U.S. EPA (1980a, 1986a). Benzidine has been widely studied for mutagenicity and genotoxicity in bacterial and yeast systems, in cultured mammalian cell systems and in rodents in vivo, and results were generally positive. It is beyond the scope of this document to review these studies here.

4.4. WEIGHT OF EVIDENCE

The evidence that benzidine is carcinogenic to humans and animals is overwhelming. Numerous epidemiological studies and case reports have demonstrated that benzidine is a human carcinogen (Zavon et al., 1973; Tsuchiya et al., 1975; U.S. EPA, 1986a). These data provided sufficient evidence to support a causal connection between exposure to benzidine and bladder cancer. In addition, the oral exposure of rats and mice to benzidine dihydrochloride induced liver and Harderian gland tumors and uterine angiomas (Littlefield et al., 1983; Firth et al., 1979, 1980; Vesselinovitch et al., 1975). According to U.S. EPA Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1986b) benzidine is a Group A human carcinogen.

5. REGULATORY STANDARDS AND CRITERIA

Many agencies, administrations and organizations consider benzidine a carcinogen (OSHA, 1984; ACGIH, 1986; NIOSH, 1978; U.S. EPA, 1980a, 1986a). Absorption through the skin contributes to the overall exposure to the chemical (ACGIH, 1986); therefore, ACGIH (1986) recommended no exposure to or contact with benzidine by any route. U.S. EPA (1980a) recommended zero exposure to benzidine based on the nonthreshold assumption, and has suggested criteria of 1.2, 0.12 and 0.01 ng/ℓ corresponding to incremental increased lifetime cancer risks of 10^{-5}, 10^{-6} and 10^{-7}, respectively. These criteria were based on the excess cancer risk to humans exposed to benzidine in the work place as estimated from the data by Zavon et al. (1973).

6. RISK ASSESSMENT

6.1. SUBCHRONIC REFERENCE DOSE (RfD_S)

6.1.1. Oral (RfD_{SO}). Benzidine is a human bladder carcinogen (Zavon et al., 1973; Tsuchiya et al., 1975; U.S. EPA, 1986a); therefore, it is inappropriate to derive an RfD_{SO}.

6.1.2. Inhalation (RfD_{SI}). Benzidine is a human bladder carcinogen (Zavon et al., 1973; Tsuchiya et al., 1975; U.S. EPA, 1986a); therefore, it is inappropriate to derive an RfD_{SI}.

6.2. REFERENCE DOSE (RfD)

6.2.1. Oral (RfD_O). Benzidine is a human bladder carcinogen (Zavon et al., 1973; Tsuchiya et al., 1975; U.S. EPA, 1986a); therefore, it is inappropriate to derive an RfD_O.

6.2.2. Inhalation (RfD_I). Benzidine is a human bladder carcinogen (Zavon et al., 1973; Tsuchiya et al., 1975; U.S. EPA, 1986a); therefore, it is inappropriate to derive an RfD_I.

6.3. CARCINOGENIC POTENCY (q_1^*)

6.3.1. Oral. U.S. EPA (1980a) derived and U.S. EPA (1986a) recommended as an interim value a potency factor (B) of 234.13 $(mg/kg/day)^{-1}$ based on the study by Zavon et al. (1973) in which 13/25 workers exposed to benzidine by inhalation developed bladder cancer. U.S. EPA (1980a) calculated the potency factor (B) by estimating that increased cancer incidence with age ($-Bd^t$) varied by the third power of age. For bladder cancer, however, the rate may increase with the sixth power of age (U.S. EPA, 1986a); thus, the q_1^* if recalculated may be approximately double (U.S. EPA, 1986a). U.S. EPA (1986a) considered this limitation and uncertainties related to exposure estimates sufficient to warrant revaluation and possibly development of a new quantitative risk estimate for benzidine.

6.3.2. Inhalation. The occupational study by Zavon et al. (1973), which served as the basis for an estimate of carcinogenic potency by the oral route (U.S. EPA, 1980a), may also be used to estimate a carcinogenic potency for inhalation exposure. In the derivation of carcinogenic potency for oral exposure, an absorbed dose of 0.0473 mg/kg/workday, was estimated based on the level of benzidine recovered from the urine of the exposed workers. This estimate was based on a value for benzidine in the urine of 0.04 mg/ℓ at the end of the workday. It was assumed that humans weigh 70 kg, produce 1.2 ℓ of urine/day and eliminate 1.45% of the absorbed benzidine as unchanged compound in the urine (Rhinde and Troll, 1974).

Zavon et al. (1973) measured concentrations of benzidine in workroom air and found levels from <0.005-17.6 mg/m³, depending on the work station evaluated. It was impossible to estimate a mean exposure level, however, because how long each individual remained at each work station or how the duties at the various work stations were shared or delegated among the workers were not reported. Assuming an inhalation absorption factor of 0.5, an inhalation "dose" of 0.0946 mg/kg/workday can be estimated by dividing the estimated equivalent absorbed dose of 0.0473 mg/kg/workday by 0.5. To estimate an equivalent absorbed dose, U.S. EPA (1980a) assumed that 240 days/year are worked and that exposure averaged 11.46 years, with an average age of 56.5 years at the end of a 13-year postexposure observation period. Applying these assumptions to the estimated inhalation dose of 0.0946 mg/kg/workday results in a TWA estimated lifetime exposure of 0.0126 mg/kg/day, as follows:

$$0.0946 \times 240/365 \times 11.46/56.5 = 0.0126 \text{ mg/kg/day.}$$

A carcinogenic potency for inhalation exposure to benzidine can be estimated by using the model $P = 1-\exp[-Bdt^3]$, which rearranged yields the following: $B=[-\ln(1-13/25)]/[0.0126 \times (56.5/70.0)^3]$, where 13/25 is the incidence of bladder cancer in exposed workers, 0.0126 mg/kg/day is the estimated average lifetime exposure to benzidine, 56.5 years is the average age of workers at the end of the 13-year observation period and 70.0 years is the reference human lifespan. The estimate of carcinogenic potency by inhalation exposure (B) is 111 $(mg/kg/day)^{-1}$.

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APPENDIX

Summary Table for Benzidine

Route	Species	Experimental Exposure/Dose (mg/kg/day)	Effect	Carcinogenic Potency $(mg/kg/day)^{-1}$	Reference
Oral	human	occupational exposure (estimated equivalent oral absorbed dose of 0.0063 mg/kg/day)	bladder cancer	234.13	Zavon et al., 1973; U.S. EPA, 1980a, 1986a
Inhalation	human	occupational exposure (estimated 0.0126 mg/kg/day inhalation dose)	bladder cancer	111	Zavon et al., 1973